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Surface Modification using Plasma treatments and Adhesion Peptide for Durable Tissue-Engineered Heart Valves

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1. Introduction

Artificial heart valves are used in valvular heart diseases, but these valves have disadvantages that they cannot grow, repair and remodel. In current study, the strategies to development of in vitro cultured functional tissue by tissue engineering is available to heart valve disease. In the point of using viable autolougous cells, tissue engineered heart valves have some advantage to include that they can repair, remodel, and grow. Because heart valve is placed under the strong shear stress condition by pumping of heart, the durability of tissue-engineered heart valves is now questionable. The purpose of the study is to evaluate of the durability of tissue engineered heart valve with surface modified scaffolds under hemodynamic conditions.

2. Materials and methods

The scaffold was constructed with poly(D,L lactid-co-glycolide) (PGLA) by salt leaching method. To enhance cell attachment and in-growth, surface properties were modified by immobilization of fibronectin and adhesion peptide sequence (RGD). For effective surface modification, the scaffolds were CO_2 gas plasma treated prior to the immobilization process for 10 min at 50W power with the pressure of CO_2 gas kept at 250 mTorr. To evaluate the chemical effect of plasma treatment, FT-IR analysis was performed. Then Scaffolds were placed in 24-well culture plate and seeded with about 3X105 vascular cells/scaffold each day for 3 days. Cell polymer constructs were cultured in cell culture medium in a humidified incubator at 37 C with 5% CO_2 for 3 weeks. The mock circulation system using a pulsatile pump was used for durability test under various shear stresses physiological condition (Fig 1). The cell-polymer construct was evaluated with MTT assays for cell proliferation.

3. Results and discussion

Scaffold Properties

Using salt leaching method, two types of scaffolds werefabricated by controlling initial weight ratio of salt/polymer. The pore size of scaffolds had a 74.4(+-0.17%) diameter, which was large enough for cells to settle on scaffolds. The porosity and pore area of PLGA 90 were much higher than those of PLGA 80. Especially total pore area of PLGA 90 was 2.7 times as large as that of PLGA 80.

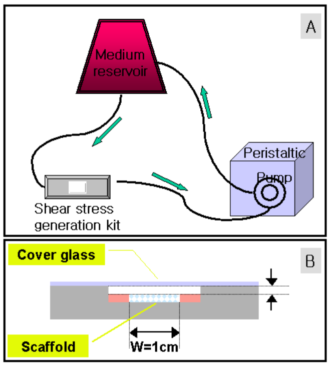

Fig. 1. schematic diagram of the shear flow experiment system (A. Overall scheme, B. A cross sectional view of shear stress generation kit)

These factors would have an influence to cell adhesion on scaffolds. Mechanical testing showed the polymer scaffolds had proper young's modulus(884.44kPa) to be sutured for formation of 3-dimensional heart valve shape. Additionally, elongation at maximum stress was 123% and ultimate tensile strength was 50.85kPa. FT-IR data shows the transmittance spectra of non-treated PLGA, plasma treated PLGA, and RGD coated PLGA after plasma treated.(Fig. 2) Transmittance peak associated with plasma treated PLGA showed a strong absorbance at 1700cm-1, which appeared in the spectra of carboxyl group by CO2 gas plasma treatment. Transmittance peak associated with RGD coated PLGA after plasma treated showed that a strong absorbance at 1700cm-1 was disappeared by RGD coating.

The effect of hydrophilic surfaces

Initial cell adhesion was evaluated by MTT result at first day after cell seeding. In adhered cell number calculated by MTT standard calibration curve,

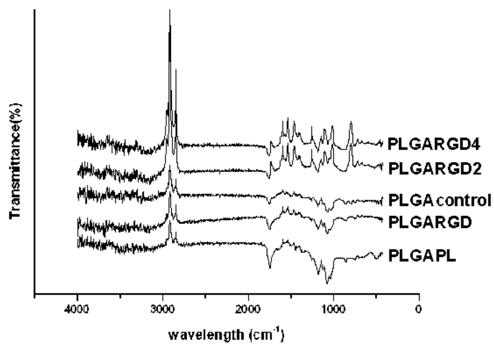

Fig. 2. FT-IR spectra. The transmittance spectra of non-treated PLGA, plasma treated PLGA, and RGD coated PLGA after plasma treated

seeding efficiency of PLGA 90(57%) is better than PLGA 80(32%). The higher porosity scaffolds had, the better cell adhesion was observed and plasma treated scaffolds had higher efficiency of cell seeding than non-treated scaffolds. But in viewpoint of cell ingrowth, while the cell number of PLGA 90 decreased, that of PLGA increased. (Fig. 3). Though 80% porous scaffolds had low cell adhesion relative to 90% ones, 2 weeks after plasma treated 80% scaffolds reached the same level of cell number by cell proliferation.

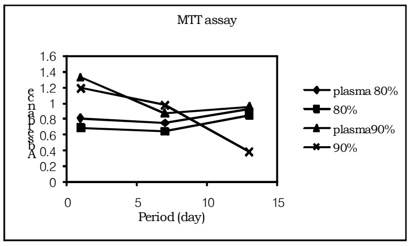

Fig. 3. Growth curve of tissue-engineered constructs on plasma treated scaffolds through MTT assay.

Effect of Fibronectin coating under shear stress conditions

MTT assay showed cell on the fibronectin coated scaffolds after plasma treatment had higher growth rate than those on only plasma treated or only fibronectin coated scaffolds. Through this result, the fibronectin coating after plasma treatment might enhance the cell ingrowth and spreading. By controlling pump flow rates, various shear stresses generated. In the case of the evaluation for the effect of plasma treatment and fibronectin coating, cell detachments of approximately 50% were observed at high shear stress condition (278dyne/cm^2) for all tissue-engineered constructs except fibronectin coated scaffolds with 0.05mg/ml after surface modification by plasma treatment. These scaffolds maintained cell attachment more than 77% at high shear stress condition (Fig. 4A). Also, Cell detachment ratio of cell-polymer constructs exposed at various shear stresses for evaluating the effect of fibronectin concentration after plasma treatment showed that optimum concentration was 0.05mg/ml (Fig.4B). Time studies showed fibronectin coated scaffolds after plasma treatment and only plasma treated scaffolds did not have significant difference for attached cell ratio(FN:85%, plasma: 76%) until 2 hours, but attached cell ratio decreased rapidly (42%) at only plasma treated scaffolds after 2 hours while they were maintained at fibronectin coated scaffolds (FN:79%) (Fig. 4C). Additionally after 6 hours, fibronecting coated scaffold with implementing plasma treatment have better results than others (Fig. 4D).

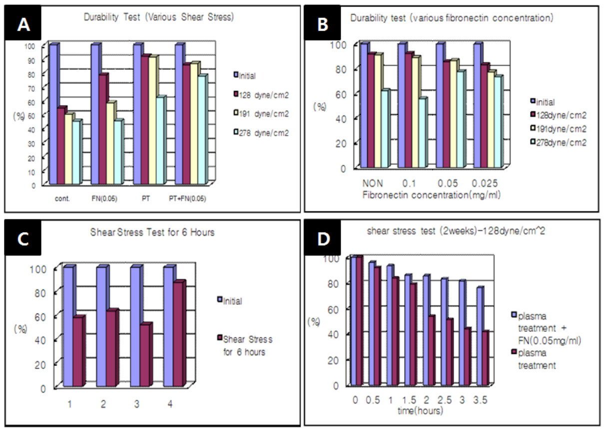

Fig. 4A, B. Cell detachment ratio of cell-polymer constructs exposed at various shear stresses (A: the effect of plasma treatment and fibronectin coating, B: the effect of fibronectin concentration), Fig. 4C,D: Cell detachment ratio of cell-polymer constructs exposed at the shear stress (C: for 4 hours - effect of fibronectin coating, D: after 6 hours – evaluation of final state)

4. Discussion

In this study, one of these techniques, plasma treatment, was used. Plasma treatment was thought that the surface characteristics were changed to hydrophilic properties. Hydrophilic conditions were known to antithrombotic conditions. After that, fibronetin was coated for enhancing the interaction between cells and polymer surface. However, in this case, interaction between fibronetin and hydrophilic surface should be considered because fibronectin is a protein. Usually most proteins have hydrophobic properties. However, in our results, combination modification with plasma treatment and fibronectin had the best result. These results were thought to result from this possibility that fibronectin is a multi adhesive protein and hydrophilic domain of it might bind with hydrophilic surface. Also, currently studies showed that tissue engineered heart valves have good performance until 6 months. But long-term studies have not yet been properly investigated. Also, in this study, short-term studies were examined. Instead, shear stress conditions were set to very severe conditions to compare with in-vivo hemodynamic conditions. In other words, normal shear stresses for long-term period were displaced with high shear stresses for short-term period. However, in this relation, reliability should be considered because the effect of peeling cells out initially by high shear stresses will pass over to normal condition.

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